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GAS-CHROMATOGRAPHIC STUDIES ON THE DISTRIBUTION OF TRITIUM IN LABELLED TOLUENE

von

H. J. Ache, W. Herr and A. Thiemann

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H. J. Ache, W. Herr and A. Thiemann

Max-Planck-Institut für Chemie, Mainz
Institut für Kernchemie der Universität Köln
and
Kernforschungsanlage, Jülich
Federal Republic of Germany

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Abstract — Résumé — Аннотация — Resumen

Gas-chromatographic studies on the distribution of tritium in labelled toluene. The distribution of tritium in tritiated toluene was measured by preparing appropriate nitro-derivates. These were separated on gas-chromatographic columns. The substances were identified by thermal conductivity measurements, simultaneously the H^3 activity was recorded by a gas counter.

For the high-boiling substances it was necessary to run the gas chromatograph and the counter at temperatures up to 220° C.

In consequence considerable analytical difficulties had to be overcome. It was found that the addition of nitrobenzene to the counting gas, which consisted of helium and methane, proved to be especially useful.

The results of various labelling methods are compared and discussed in the light of current research.

Etude de la distribution du tritium dans le toluène marqué, par chromatographie gazeuse. Les auteurs ont mesuré la distribution du tritium dans le toluène tritié en préparant des dérivés nitrés appropriés qui ont ensuite été séparés dans des colonnes pour chromatographie gazeuse. Les différentes substances ont été identifiées par des mesures de conductivité; en même temps, l'activité du ^3H était enregistrée par un compteur à gaz.

Pour les substances à point d'ébullition élevé, il a fallu faire fonctionner le chromatographe à gaz et le compteur à des températures atteignant 220° C.

Il a donc fallu aussi surmonter par la suite des difficultés d'analyse considérables. L'addition de nitrobenzène au gaz de comptage, composé d'hélium et de méthane, s'est révélée particulièrement utile.

Les auteurs comparent et examinent les résultats des différentes méthodes de marquage, compte tenu de l'état présent des recherches.

Изучение распределения трития в меченом толуоле при помощи метода газовой хроматографии. Распределение трития в тритиесодержащем толуоле измерялось путем приготовления соответствующих азотопроизводных. Они разделялись на газохроматографических колонках. Вещества определялись измерением проводимости с одновременным регистрированием активности H^3 на газовом счетчике.

Для веществ с высокой точкой випения необходимо, чтобы газовый хроматограф и счетчик работали при температуре до 220° C.

Ввиду этого надо было преодолеть значительные аналитические трудности. Было найдено, что примесь нитробензола в находящемся в счетчике газе, состоящем из метана и гелия, оказалась особенно полезной.

Результаты различных методов мечения сравниваются и обсуждаются в свете современного опыта.

Estudio mediante cromatografía en fase gaseosa de la repartición del tritio en el tolueno marcado. Los autores prepararon nitroderivados que les han permitido medir la distribución del tritio en el tritiado. Separaron los nitroderivados en una columna de cromatografía en fase gaseosa e identificaron las sustancias por mediciones de la conductividad; al mismo tiempo registraron la actividad del ^3H mediante un contador de gas.

La cromatografía y el recuento de las sustancias de elevado punto de ebullición tuvieron que llevarse a cabo a temperaturas de hasta 220° C. Por consiguiente, fue preciso resolver diversos problemas de orden analítico. Los autores encontraron que es muy conveniente añadir nitrobenceno al gas de recuento, consistente en una mezcla de helio y metano.

Los autores comparan y discuten los resultados de los diversos métodos de marcación, teniendo en cuenta el estado actual de las investigaciones.

The direct labelling of organic molecules with tritium can be carried out in two different ways. On the one hand, highly energetic recoil tritons produced by the Li^6 (n, α) T or He^3 (n, p) T nuclear reaction may, because of their high kinetic energy, break the C—H or the C—C bonds and may finally combine with the rest of the molecule [1]. On the other hand, there is the so-called WILZBACH labelling process [2]. Here the beta-decay gives rise to recoiling tritium ions and also leads to excitation and ionization, which favour substitution reactions. Obviously this process can be promoted by external gamma-radiation, ultraviolet radiation, electric discharge and even by micro-wave energy-transfer [3]. The

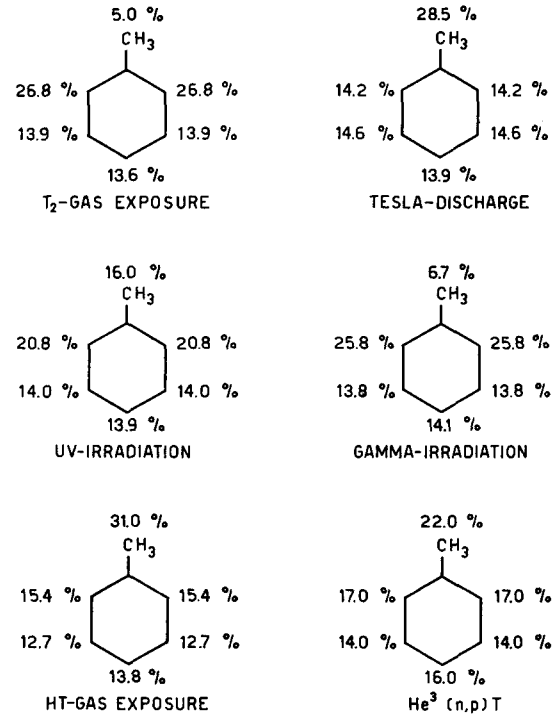


Fig. X
Intramolecular distribution of tritium atoms

reaction mechanism of the labelling process has been studied in the course of recent years by several authors. Here, studies of the methane system and of other saturated and unsaturated lower hydrocarbons proved to be the most successful [4]. The results of these studies proved the reaction mechanism involved to be fundamental and applicable also to more complex systems. However, with larger molecules the knowledge of the ions present, their state of excitation and their reactivity is so fragmentary that the assignment of a clear and detailed reaction path for the labelling process is very difficult. Now it is remarkable that in practically all the investigations mentioned, only the tritium activity of the labelled products and the by-products was studied but little, if any, information was obtained about the distribution of tritium in the molecules themselves. We were accordingly interested to learn more about the influence of the labelling method and of experimental conditions on the final distribution of the tritium atoms in a complex molecule.

The toluene molecule seemed to us a useful model. We tritiated this substance by various methods, as described below. It is worth mentioning that the radiometric analysis of the various by-products such as benzene, acetylene, etc., showed no qualitative but only quantitative differences. For example, the amount of active by-products obtained by the electric discharge-method was much higher than that found on using the gas-exposure technique. The detailed analysis of these substances, however, was felt to be of secondary importance. The emphasis in our studies was on the behaviour of the tritium-atoms in the toluene molecule, e.g. by investigating the intramolecular distribution. In Fig. X our results are summarized.

Experimental conditions

(1) WILZBACH-LABELLING

Gas-chromatographically purified toluene and 2 c of pure tritium gas were sealed in glass ampoules (Fig. 1). The volume of the ampoules was 50 ml, the toluene pressure 24 mm Hg, temperature 25 °C, and the calculated weight of the toluene was ~6 mg. After an irradiation of 10 curie-days the tritium was removed and the active toluene was purified on a gas-chromatographic column in order to obtain high radiochemical purity. Afterwards it was diluted with inactive toluene.

(2) LABELLING BY TESLA-DISCHARGE

For labelling by electric discharge we used the glass vessel shown in Fig. 2. Its volume was 50 ml, the distance between the electrodes 20 mm and its diameter 20 mm. One electrode was grounded, the second was connected to a normal Tesla leak-detector. The voltage applied was about 3000 V. The toluene gas pressure was 24 mm Hg, the amount of tritium used was 0.2—2.0 curies. Irradiation time varied from 5 to 30 minutes. Purification of the tritiated toluene was carried out in the usual manner by gas chromatography.

(3) LABELLING BY UV-IRRADIATION

A mixture of 0.2 c tritium and toluene gas (24 mm Hg, 25 °C) was irradiated under a high-pressure quartz lamp (300 W) in cylindrical quartz ampoules of 50 ml. A blank run under the same conditions (without UV-light) yielded only ~50% of the H^3 activity incorporated in the toluene.

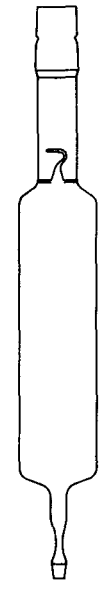


Fig. 1
Irradiation vessel

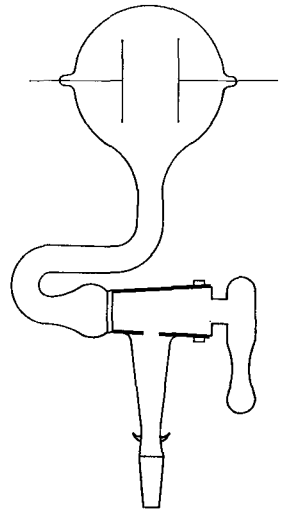


Fig. 2
Tesla discharge cell

(4) LABELLING BY GAMMA-IRRADIATION

Samples of 0.2 c tritium and 300 ml toluene gas (24 mm Hg) were irradiated in glass bulbs (100 ml volume) for 7 days using a Co^{60}-source (1.5×10^5 r/h, total dose 2.8×10^7 r, 25 °C).

(5) LABELLING BY THE He_i^3 (n, p) T PROCESS

Several quartz ampoules 20 ml in volume were filled with toluene gas (24 mm Hg) and He^3. The ampoules were irradiated at a neutron flux of 3×10^{11} n/cm² sec. for 8 hours. The gamma-dose was $\sim 5 \times 10^4$ r/h.

(6) LABELLING BY HT-EXPOSURE

1 c tritium (about 0.4 ml at 760 mm Hg) was mixed with a larger amount of hydrogen. This gas mixture was subjected to an electric discharge from a Tesla leak-detector for 30 minutes. The resulting HT gas was sealed in a glass bulb with 100 ml toluene gas for 10 days.

Method of measurement

The position of the tritium in a labelled toluene molecule can be determined by stepwise degradation to the corresponding derivatives. This method is without doubt very accurate but also tedious and time-consuming, since five different derivatives were to be synthesized and purified. We therefore worked out a different method which provided us with faster results of comparable accuracy. We used radio gas chromatography to analyse toluene and its mono-nitro derivatives (*ortho-*, *meta-*, and *para*-nitrotoluene). The following procedure was used.

The irradiated toluene is gas-chromatographically purified. One part is put aside, and the rest is treated with a mixture of nitric and sulphuric acid: 3 g toluene is added drop by drop to a mixture of 3 g HNO_3 (d = 1.4) and 4 g H_2SO_4 (d = 1.98) at 60 °C. The mixture is then cooled and diluted with ice-water. The nitrotoluene is extracted with ether, neutralized, washed and dried over $CaCl_2$. The ether is removed on a water-bath, the unreacted toluene is distilled off and discarded. After this the tritiated toluene, which was kept in reserve, is added and the mixture of toluene, *ortho-*, *meta-*, and *para*-nitrotoluene is separated and analysed by radio gas-chromatography. One of the main requirements of this method is that no isotope effects and exchange reactions take place. The investigations of MELANDER [5] on dinitrotoluenes showed that isotope effects are not observed in the nitration of tritium- and deuterium-labelled toluene. $^3H^+$ reacts in the same unspecific manner as $^1H^+$, when replaced by NO_2^+. We made sure that this was also the case in the mononitration of the toluene-molecule. We prepared *ortho*-T-toluene by the reaction of the Grignard-compound of *ortho*-bromo-toluene with T_2O-containing water; this *o*-T-toluene was mononitrated and analysed. The three nitrotoluenes ($o:m:p = 62:5:33$) were found to contain the calculated activity; *meta*-T- and *para*-T-toluene showed the same results. In our radio gas-chromatographic work we used a normal apparatus connected to a proportional gas flow-counter (Fig. 3). Both instruments recorded simultaneously. The 8-m separating-column had an inner diameter of 8 mm. The filling-material was fire-brick powder (0.3—0.4 mm) and the liquid phase 2.4.7.-trinitrofluorenone. Helium was used as carrier-gas. The flow-counter (Fig. 4) is similar to that described by WOLFGANG and ROWLAND [6]. These authors operated their counter at temperatures up to 150 °C with a counting-gas mixture of argon/butane or helium/methane. As a matter of fact we were not able to work with these gas mixtures because we had to use higher temperatures and the nitrocompounds so badly poisoned and altered the counting-properties that inactive samples gave "negative" peaks. We tackled this problem by adding a constant percentage of the "poison" to the

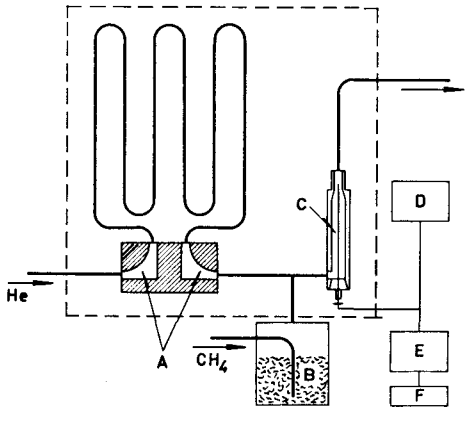


Fig. 3
Gas-chromatography apparatus
A: bridge circuit; B: nitrobenzene chamber; C: counter; D: 5000 V supply; E: high-gain amplifier scale; F: rate-meter

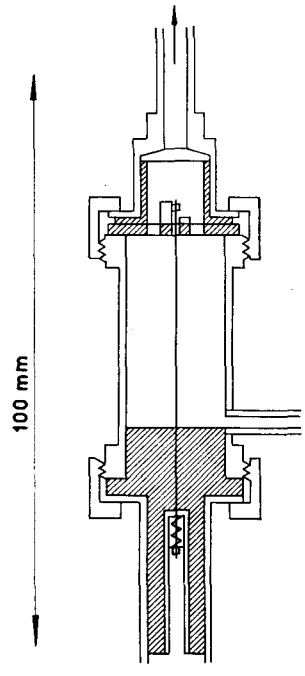


Fig. 4
Internal vapour flow-counter

counting gas, so maintaining constant counting properties. It was not necessary to add the same substance; a chemically similar compound worked equally well. We finally selected a gas-mixture of 62 vol. % helium as carrier-gas, 34 vol. % methane as quenching-gas and 4 vol. % nitrobenzene vapour. With a counting gas of this composition larger samples, up to 10 mg of nitrobenzene or nitrotoluene, could be measured without trouble (Fig. 5).

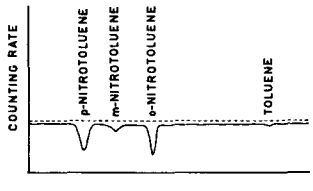


Fig. 5
Change in counting rate obtained with an external source on addition of an inactive mixture of toluene and nitrotoluene
----- helium-methane nitrobenzene mixture
——— helium-methane mixture, no nitrobenzene added

It is worth mentioning that some other high-boiling substances, such as chlorobenzene and chlorotoluene could be analysed in a similar way by adding chlorobenzene vapour to the counting gas. Figs. 6 and 7 show that increasing amounts of tritiated nitrotoluene as well as of toluene give a counting rate nearly proportional to the amount used. Working at a temperature of 210 °C we found a

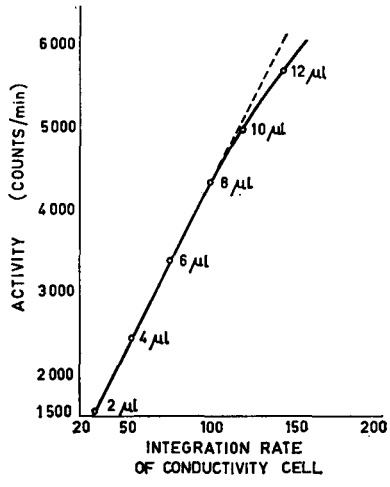


Fig. 6
H^3-activity against amount of injected toluene

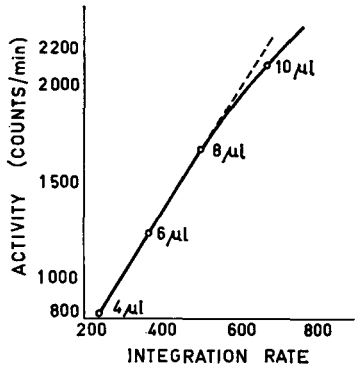


Fig. 7
H^3-activity against amount of injected *o*-nitroluene

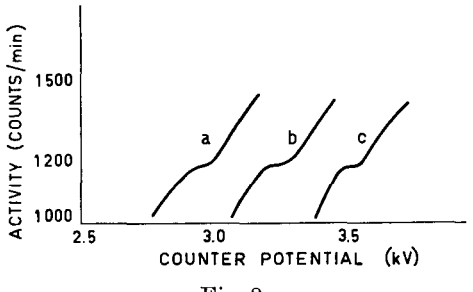


Fig. 8
Counter plateau curves showing helium-methane-nitrobenzene mixtures as a function of nitrobenzene concentration
a: 2% nitrobenzene; b: 4% nitrobenzene; c: 6% nitrobenzene

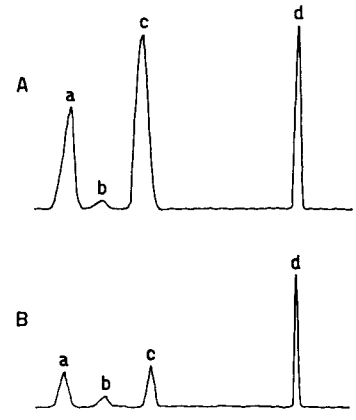


Fig. 9
Gas chromatography of a mixture of toluene and the mono-nitro toluenes
A. Gas conductivity record
B. H^3-activity record
a: *p*-nitrotoluene; b: *m*-nitrotoluene; c: *o*-nitrotoluene; d: toluene

plateau of about 50 V (Fig. 8). The background could be kept to 40 counts/min. The flow-rate was 40 ml of He and 100 ml methane/nitrobenzene per minute. Samples of 10^{-4} μC H^3 could still be measured accurately in this way. Molar specific activities were determined by comparing test-samples of known composition; these were needed to calculate the tritium content of the separated compounds concerned.

The results of a gas-chromatographic separation are shown in Fig. 9.

Discussion of the results

Only three years ago at the Symposium on Tritium in New York Wilzbach reported on the tritium distribution in the toluene molecule following gas exposure. With the limits of experimental error we can confirm his values. Furthermore, we are now able to compare the different labelling methods with each other.

There is an obvious difference in the tritium content of side-chain and *ortho*-position of toluene induced by the normal Wilzbach process and by electric discharge. We may draw the conclusion that here a somewhat different substitution mechanism is involved. The experiments of WOLFGANG *et al.* [7] and also of GANT and YANG [8] on the Wilzbach-labelling of methane and ethylene showed that the mechanism of the reaction of T_2 and CH_4 and C_2H_4, respectively may in general follow three main lines.

(1) DECAY-INDUCED MECHANISM

$$T_2 \rightarrow He^3T^+ + \beta^-$$
$$He^3T^+ + CH_4 \rightarrow CH_3T$$

(2) EXCHANGE OF TRITIUM WITH A RADIATION-PRODUCED ION

$$CH_4 \rightarrow ion^+ + e^-$$
$$ion^+ + T_2 \rightarrow CH_3T$$

(3) EXCHANGE OF A RADIATION-ACTIVATED TRITIUM SPECIES

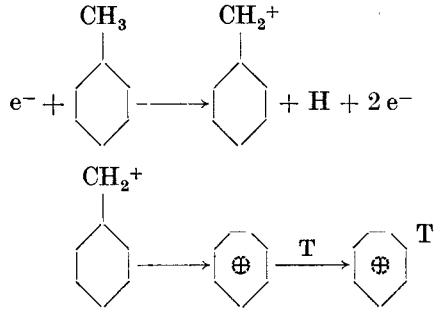
The mechanism taking place during the Tesla discharge seems to be rather complicated and difficult to analyse. However, it may be taken for granted that the organic molecules as well as the tritium molecules are excited and ionized by the Tesla discharge. Therefore, the second and third reactions shown above take place preferentially. It is obvious that in the toluene molecules the decay-induced mechanism during the Tesla discharge is relatively unimportant because of the short time available.

We find most of the activity located in the side chain. The experience gained with normal chemical reactions indicates that the side chain seems to be mainly replaced by a radical mechanism. The idea suggests itself that the substitution of the toluene via Tesla discharge is the result of reactions of highly excited molecules, as indicated by the following steps:

$$T + C_6H_5CH_3 \rightarrow C_6H_6 + CH_2T$$
$$T + C_6H_5CH_3 \rightarrow C_6H_5T + CH_3$$
$$CH_3 + CH_2T + C_6H_6 \rightarrow C_6H_5CHT + CH_4$$
$$2\,CH_3 + C_6H_5T \rightarrow C_6H_5TCH_2 + CH_4$$

We believe that this reaction chain can best explain the remarkably equal distribution of tritium in the aromatic ring and the strong preference for the side chain.

An alternative explanation could be given if we assume that the reaction is taking place via a symmetrical intermediate of the formula $C_7H_7^+$, so that an equal distribution of the activity among the seven H-positions would be obtained. Such an intermediate would be the already known tropylium ion, which was found by RYLANDER, MEYERSON and GRUBB [9] by mass-spectrometric investigations of ionized toluene. The process of substitution could then be written as follows.



The question whether isomerization is possible in the toluene molecule under the stress of Tesla discharge was also positively answered in our experiments. We synthesized *m*-T-toluene via a Grignard reaction with T_2O-containing water; the compound was then subjected to a Tesla discharge for 2 hours. In addition to hydrogen and lower hydrocarbons we found benzene, ethylbenzene and xylene as decomposition products. Tritium formerly located in the *meta*-position was found after the treatment in the other positions, too (Fig. Y). These results appear to indicate the existence of a symmetrical intermediate. The isomerization of 7-T-toluene by gamma-irradiation studied by WILZBACH [10] may lead to the same conclusion.

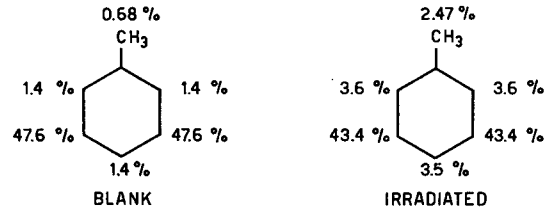


Fig. Y
Meta-T-toluene irradiated by Tesla-discharge

The fact that toluene labelled by the gas-exposure method shows a different distribution of tritium makes it probable that here the symmetrical intermediate is bypassed, and we think that the reaction $T_2 \rightarrow (^3HeT)^+ + \beta^-$ is mainly responsible. Consequently, a minor part of the molecules will be labelled by normal beta-radiolysis.

The high-energy recoil tritons from the processes Li^6 (n, α) T and He^3 (n, p) T have to cool down before being able to react; during this time they may lose their charge. On the other hand, the $(He^3T)^+$ ions originating from the T_2-β^- decay have comparatively low kinetic energy and may keep their charges until reaction. Therefore it is most likely that they may obey in some way the substitution rules of organic chemistry, as they are valid for aromatic molecules.

The toluene molecule is comparatively symmetrical. Its dipole-moment is only 0.41 D. It is difficult to observe resonance properties, as for example, hyperconjugation. The distance of the methyl carbon from the ring carbon is that of a normal C—C bond. However, on nitration and other chemical reactions a remarkable preference for the *ortho*-position can be observed, indicating that a centre of negative charge is located there. That substitution in the *ortho*-position is preferred can be seen from the gas-exposure experiment (Fig. X). Other positions in the ring are not so characteristic.

In confirming our investigation of this problem we tried to find out what part of the labelling was due to a beta-radiolysis process; we excluded the decay-induced mechanism by using the tritium in the form of HT. The reaction $T_2 \rightarrow (HeT)^+ + \beta^-$ can no longer take place; labelling is only possible by beta-radiolysis. The activity obtained in this way was only about 10% of that measured after comparable T_2 gas-exposure experiments. It appears from the experiments that the distribution of tritium in the molecule after HT gas-exposure is very different from that observed after normal Wilzbach labelling, but, as we expected, it is similar to the tritium distribution after the Tesla-discharge treatment. In the latter a decay-induced mechanism may have been overlooked. It is too early, however, to come to definite conclusions, as our investigations are still in progress. Recent experiments showed, however, a dependence of the tritium-distribution in the molecule on the amount of tritium used, which makes additional considerations necessary.

In addition, we studied a labelling procedure using ultraviolet light and gamma-irradiation. With the first method we found 16% tritium activity in the methyl group. A blank run showed only half of the total tritium activity. Consequently, $\sim$50% of the activity originates from ultraviolet excitation, the rest from normal Wilzbach-labelling.

The result leads to the conclusion that UV-induced labelling is also comparable to the Tesla discharge mechanism. The activity incorporated after gamma-irradiation was only 1.05 times greater than in the unirradiated blanks.

High-energy recoils

The investigations of ROWLAND and co-workers [11] on substituted benzoic acids have shown that the larger portion of tritium generated by the Li^6 (n, α) T-process is incorporated in benzoic acid. The position of tritium was favoured by exchange with the substituting group. On the other hand, distinct promotion of the *ortho*-position is conceivable. It was concluded that the amount of H^3 activity found in benzoic acid depends to a certain degree on the strength of the chemical bond of the former substituent, which proves that this substitution occurs at relatively low energies. In addition to chemical bond energy some other factors, for example the formation of reactive intermediates, their lifetime, and steric factors may influence the substitution-reaction, so that the discussion of the results of the He^3 (n, p)-T-process in toluene becomes difficult. Nevertheless, we would like to point out that our experiments were done in the gas phase and not, like Rowland's experiments, in the solid phase. The rearrangement of excited intermediates is more likely in the solid than in the gas phase because of the cage effect. Consequently a statistical distribution should rather be predominant in the latter case. We observed in this experiment a distribution which is statistical in the first approximation. If we could better exclude the contribution of the accompanying gamma-irradiation, we might suppose that there could be a certain preference for the free rotating methyl group. Further systematic investigation may provide us with more detailed information.

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